Amendments to the Specification:

Before the first paragraph on page 1, please insert the following:

The instant application is a national stage 371 application of PCT/GB99/02205 having an International Filing Date of July 9, 1999 and claims priority from United Kingdom Application Number 9814902.4, filed July 10, 1998.

Before the first paragraph on page 1 and after insertion of the above paragraph please add the title:

FIELD OF THE INVENTION

Before the second full paragraph on page 1 please add:

SUMMARY OF THE DISCLOSURE

After the second paragraph on page 9 please add the following:

BRIEF DESCRIPTION OF THE DRAWINGS

Prior to the paragraph beginning with "SEQIDNO1" on page 9, please insert the following:

DETAILED DISCLOSURE

Please amend the last paragraph of page 12 and extending to the top of page 13 as follows:

FIC was chosen for further characterization and silver staining of SDS-gels showed that it contains four distinct protein bands (not shown). Rabbit polyclonal antibodies were raised to eluted FIC proteins and used to screen an already established genomic expression (λ Zap II) library. Several reactive meningococcal polypeptides and their respective DNA fragments were isolated. Two of the most promising ones (TspA and TspB) were further studied. The DNA fragments were sequenced and with help from the Sanger-released genomic sequences which were produced by the Neisseria Meningitidis Sequencing Group at the Sanger Centre. ~~Group at the Sanger Centre and can be obtained from ftp://ftp.sanger.ac.uk/pub/AAREADME.release-policy.txt, the genes encoding these two proteins~~ The genes encoding these two proteins were then constructed (see SEQIDNO1-4) and cloned into high expression vectors.

Please amend the first full paragraph of page 10 as follows:

Briefly, PBMCs were isolated from heparinised blood samples by centrifugation over an aseptically filtered solution for human mononuclear cell separation. One such solution is sold under the tradename Histopaque® and is commercially available from Sigma-Aldrich Corp., having place of business in St. Louis, MO, USA. ~~histopaque (Sigma).~~ The PBMCs were washed and cultured in 96-well tissue culture plates at 2 x 10^5 cells/well in RPMI medium containing 10% human AB serum (RPMI-AB). Meningococcal proteins (from strain SD, B:15:P1,16) were prepared by growing the organism under iron restriction, to induce the expression of iron-regulated proteins which are also expressed ~~*in vivo.* [Ala'Aldeen, 1994]~~ *in vivo*. Such as described by Ala'Aldeen, D. et al., 1994. "Immune response in man and animals to meningococcal transferring-binding proteins: implications for vaccine design", Infect. Immun. 62:2894-2900, (hereinafter Ala'Aldeen, 1994). The meningococcal proteins (SD-), antigens from *Candida albicans* (a recall antigen) or phytohaemaglutinin (PHA, positive control) were added to quadruplicate wells. RPMI-AB alone (with no antigen) was added to quadruplicate wells to serve as the background. After five days all cultures were pulsed with 1μCi of tritrated thymidine and incorporation of thymidine was determined after another eighteen house. A positive response was defined as PBMC proliferation index of at least 2 (See Fig. 1).

Please amend the first full paragraph on page 11 as follows:

T-cell clones are defined here as the population of cells which originate from a single T-cell. Single T-cell receptors (TCRs) cane engage with an extraordinary small number of peptide-HLA complexes (<10/cell) as shown in "Serial triggering of many T cell receptors by a few peptide-MHC complexes," Valitute et al., Nature 1995; 375: 148-151, hereby incorporated by reference, ~~[Valitute, 1995]~~, therefore T-cell clones will provide a highly sensitive system by which it will be possible to detect the presence of peptide antigens within mixtures of proteins. T-cell lines, specific to meningococcal antigens, were seeded at 0.3 cell/well in 96-well tissue culture plates in the presence of irradiated (non-proliferating) autologous EBVB feeder cells, plus low doses of IL-2 as reported in "Selection of T cell epitopes and vaccine engineering." Sinigaglia et al., Methods in Enzymology 1991; 203: 370-386, hereby incorporated by reference. ~~[sinigaglia, 1991]~~. Cell growth was detected microscopically after one-two weeks and growing cells expanded further by stimulation with meningococcal proteins. All T-cell lines and clones were assessed for the phenotype (and ascertained to be $CD4^+$ T-cells), using flow cytometry and CD4, CD8 and α/β^- and γ/δ^- TCR-specific monoclonal antibodies. Their specificity to meningococcal proteins was tested by measurement of tritiated thymidine incorporation in response to stimulation with meningococcal proteins compared to irrelevant antigens e.g. tetanus toxoid. Large numbers of T-cell lines, oligoclones and clones from patients and normal donors have been identified and stored in liquid nitrogen until further use.

Please amend the third full paragraph of page 14 as follows:

This method had been successfully applied in other organisms to identify helper T-cell epitopes as disclosed in "Identification of a CD4+ T cell stimulating antigen of a pathogenic bacteria by expression cloning." Sanderson et al., J Exp Med 1995; 182: 1751-1757 and in

"Expression cloning of a protective leishmania antigen." Mougneau et al., Science 1995; 268: 563-566 each of which are hereby incorporated by reference in their entirety. ~~epitopes [Sanderson, 1995; Mougneau, 1995].~~ Briefly, we used an existing λZapII phage library expressing genomic DNA extracted from strain SD *N. meningitidis* ~~[Palmer, 1993 #214]. The library~~ and is disclosed in "Neisseria meningitides transferring-binding protein 1 expressed in Escherichia coli is surface exposed and binds human transferring." Palmer et al., FEMS Microbiol Lett 1993; 110: 139-146, hereby incorporated by reference. The library contains 2 x 10^5 recombinants with an average size of insert of 2.3 kb (range up to 10 kb). A representative pool of recombinant pBluescript SKII plasmid were excised (*in vivo*) from the phage library and transformed into *E. coli* strain XL1-Blue, using ExAssist helper phage (Stratgene) as described ~~previously [Ala'Aldeen, 1996; Palmer, 1993]~~. in "Cloning, sequencing, characterisation and implications on vaccine design of the novel dihydrolipoyl acetyltransferase of neisseria meningitidis." Ala'Aldeen et al., J Med Microbiol 1996; 45: 419-432 and Palmer, 1993 supra.

Please amend the first full paragraph of page 16 as follows:

One of the novel lytic bacteriophages is Novagen's T7Select Phage Display System which is easy to use and has the capacity to display peptides up to 1200 amino acids, equivalent to 3.6 kb, with protein molecular weight over 100kDa. Such high molecular weight proteins are usually expressed at low copy numbers by T7Select1. Phage T7Select415, however, is capable of displaying up to 415 copies of a peptide up to 50 amino acids in size. Phage assembly occurs in the *E. coli* cytoplasm and mature phages are release by cell lysis. The latter process occurs within a few hours of infection, which makes the system very rapid. To create a genomic display library, meningococcal DNA will be fragmented to appropriate sizes and cloned and packaged into both T7Select1 and T7Select415 vectors as described in the Novagen's T7Select System ~~manual [Novagen, 1996]. This~~ manual. This dual approach allows for the screening for both large and small polypeptides.

Please amend the third full paragraph beginning on page 16 and extending to page 17 as follows:

Another method according to the present invention involves the use of proteins and small peptides on a modified lambda capsid protein D. This protein, which is of 11 kDa with 405 copies expressed as trimers on the phage ~~head [Sternberg, 1995; Mikawa, 1996], is capable~~ head is capable of an efficient display of foreign peptides that are fused to its amino- or ~~carboxy-termini [Mikawa, 1996]. This~~ carboxy-termini and are disclosed in "Display of peptides and proteins on the surface of bacteriophage lambda." Sternberg et al. Proc Natl Acad Sci USA, 1995; 92: 1609-1613 and "Surface display of proteins on bacteriophage lambda heads." Mikawa et al. J Mol Biol 1996; 262: 21-30, both of which are hereby incorporated by reference. This system was successfully used to display a Hepatitis C genomic cDNA ~~library [Alter, 1995] and~~ library and, more recently, to generate a randomly amplified genomic PDL of known ~~organisms [Lambert, 1993; Kwong-Kowk, 1996; Tomei, 1993]. This~~ organisms. This involves generating randomly amplified DNA fragments of a known DNA template, using short (random) oligonucleotide primers in polymerase chain reaction (PCR). We have recently constructed a meningococcal genomic lambda phage display library by cloning randomly amplified PCR products in λpRH825 vector, using two random primers, each tagged at 5' end to SpeI or NotI restriction sites to facilitate insertion into the predigested vector. Packaging amplified and digested DNA fragments into lambda phage was performed using a lambda packaging kit (Pharmacia Biotech) and plated by infection of the *E. coli* strain BB4. This yielded 5 x 10^7 plaques, of which a sample of 100 pfu were randomly chosen, and their DNA inserts sequenced. Sequence alignment of the obtained sequence data with those available for *N. Meningitidis* ~~(Sanger, Wellcome)~~ and/or *N. Gonorrhoea*, confirmed that all the chosen plaques contained DNA fragments of meningococcal origin. The fragment sizes ranged from 100-200 bp, representing deduced peptides of up to 60 amino acids long. This PDL was prepared and established in IRBM for use in the identification of $CD4^+$ T-cell stimulating recombinant peptides, using the same cloning technique described for the λZapII phage system.

Please amend the first full paragraph on page 18 as follows:

In order to identify and then characterise core epitopes of each candidate peptide, progressively smaller fragments of the DNA will be cloned, expressed and further examined for T-cell stimulation. To define epitopes more accurately, short overlapping peptides representing the defined T-cell stimulating subunits are synthesised and re-examined. Then N- and C-terminal truncated analogs of the most immunogenic peptide fragment are synthesised and tested likewise. Finally, alanine scanning mutational analysis will be employed to identify critical amino acid positions responsible for both TCR contact and HLA-class II contact. Here, a series of peptide analogs of the core epitope identified in after N- and C-terminal truncation are synthesised, each with single alanine substituted at successive amino acid positions, and effects on T-cell immunogenicity and on HLA-binding ~~are assessed [Nelson, 1996]. The~~ are assessed. The isotype of class II HLA molecule restriction specificity will be identified for each T-cell clone by antibody blocking experiments.

Please amend the first full paragraph on page 19 as follows:

A central aim is to identify T-cell immunogens of *N. meningitidis* which will stimulate T-cell help for the production of protective anit- meningococcal antibodies. Having identified dominant T-cell antigens amongst the proteins, their ability to stimulate T-cell help for antibody production is invesitigated *in vivo* in animals and in an *in vitro* immunisation system which has been established and optimised in our laboratories and is disclosed in "Simulation of human B cells specific for Candida albicans for monoclonal antibody production." Davenport et al. FEMS Microbiol Immunol 1992; 89: 335-344. ~~[Davenport, 1992]. Protein fragments~~ Protein fragments of peptides that stimulate T-cells from individuals covering a range of HLA types are studied for the presence of B-cell epitopes. If the protein contains B-cell epitopes then anitbodies from individuals naturally immune to meningococcal disease should recognise these proteins in immunoblots or ELISA. If no B-cell epitopes are recognised then the identified T-cell epitopes

will be conjugated to previously characterised B-cell immunogens such as meningococcal capsular polysaccharides, the class (1, 2/3) proteins, the transferrin binding protein … etc.